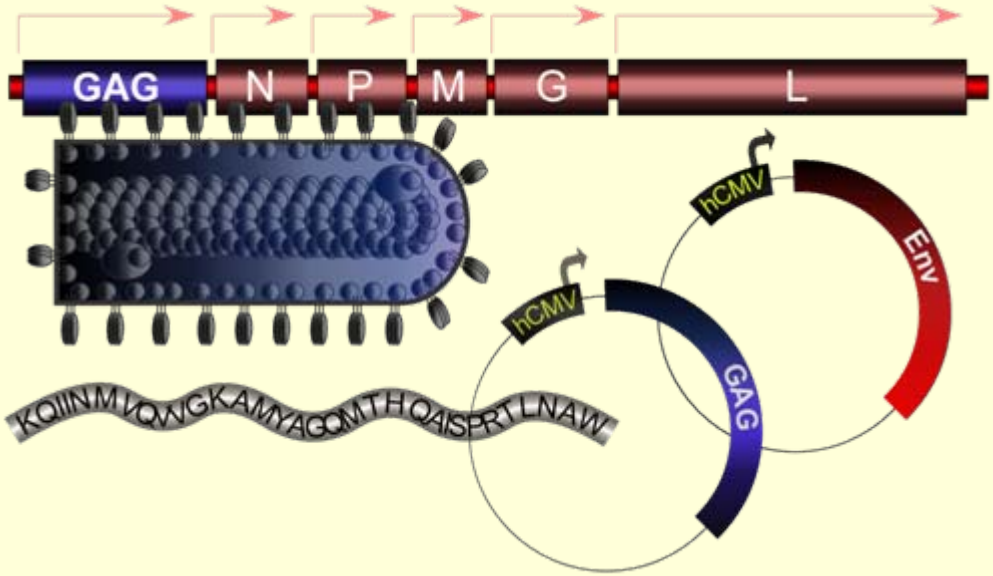
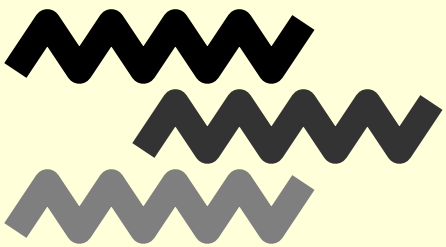
Potency Assay Development For HIV Vaccines

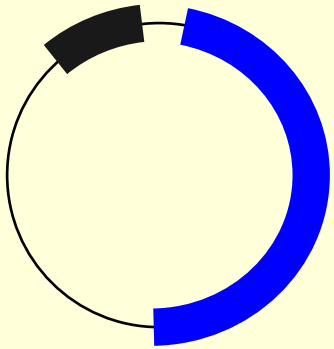


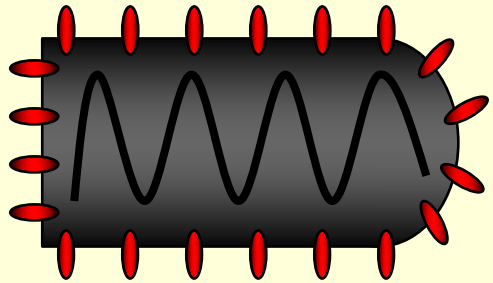
Stephen Udem, M.D., Ph.D.
Vice President
Wyeth Vaccines Discovery

Wyeth
Research

Wyeth Vaccines HIV Program





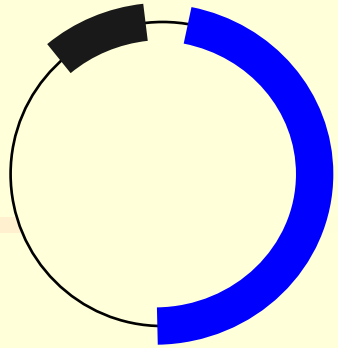


Th-CTL Peptides **Plasmid DNA** **Viral Vector**

- **Evaluate multiple vaccine delivery modalities and identify those that elicit the most robust and balanced cellular and humoral immune responses**
- **Optimize those that show the most promise**
- **Exploit heterologous prime-boost possibilities**

Wyeth Research

Plasmid DNA Vaccines



Historically

Work well in the mouse model

- **Immunogenic**
- **Provide excellent protection in challenge models**

Work less well in non-human primates

Disappointing immunogenicity in clinical trials

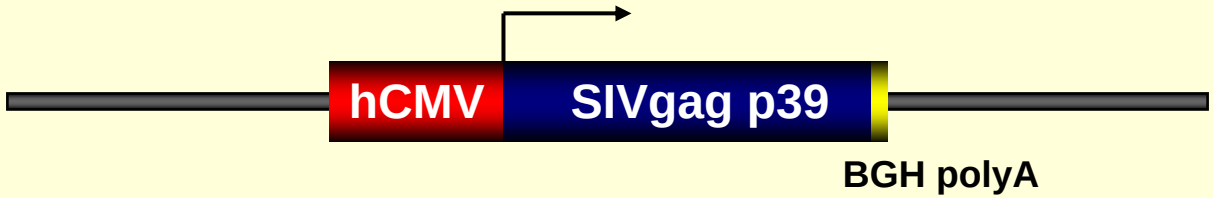
Investigating

- ***Molecular adjuvants - IL-12 and IL-15***
- ***pDNA vector modifications***
- ***Vaccine composition***

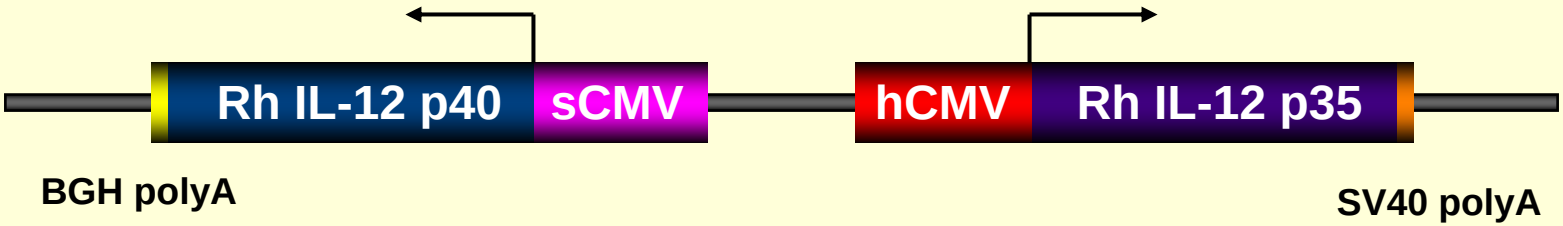
Wyeth Research

First Generation pDNA Vaccine

RNA optimized SIV *gag* p39

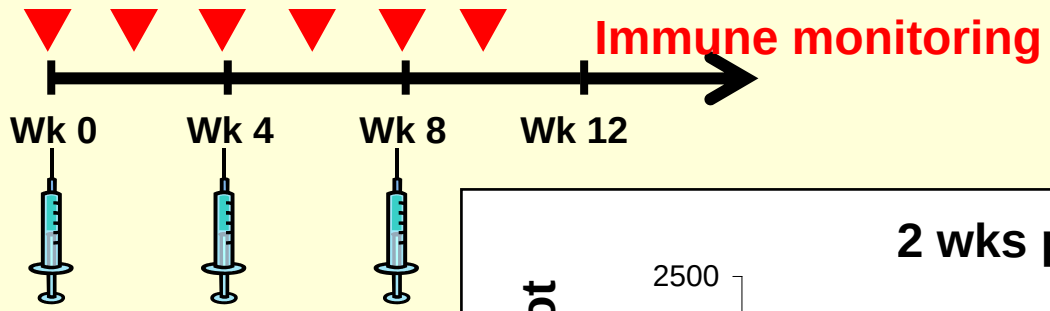


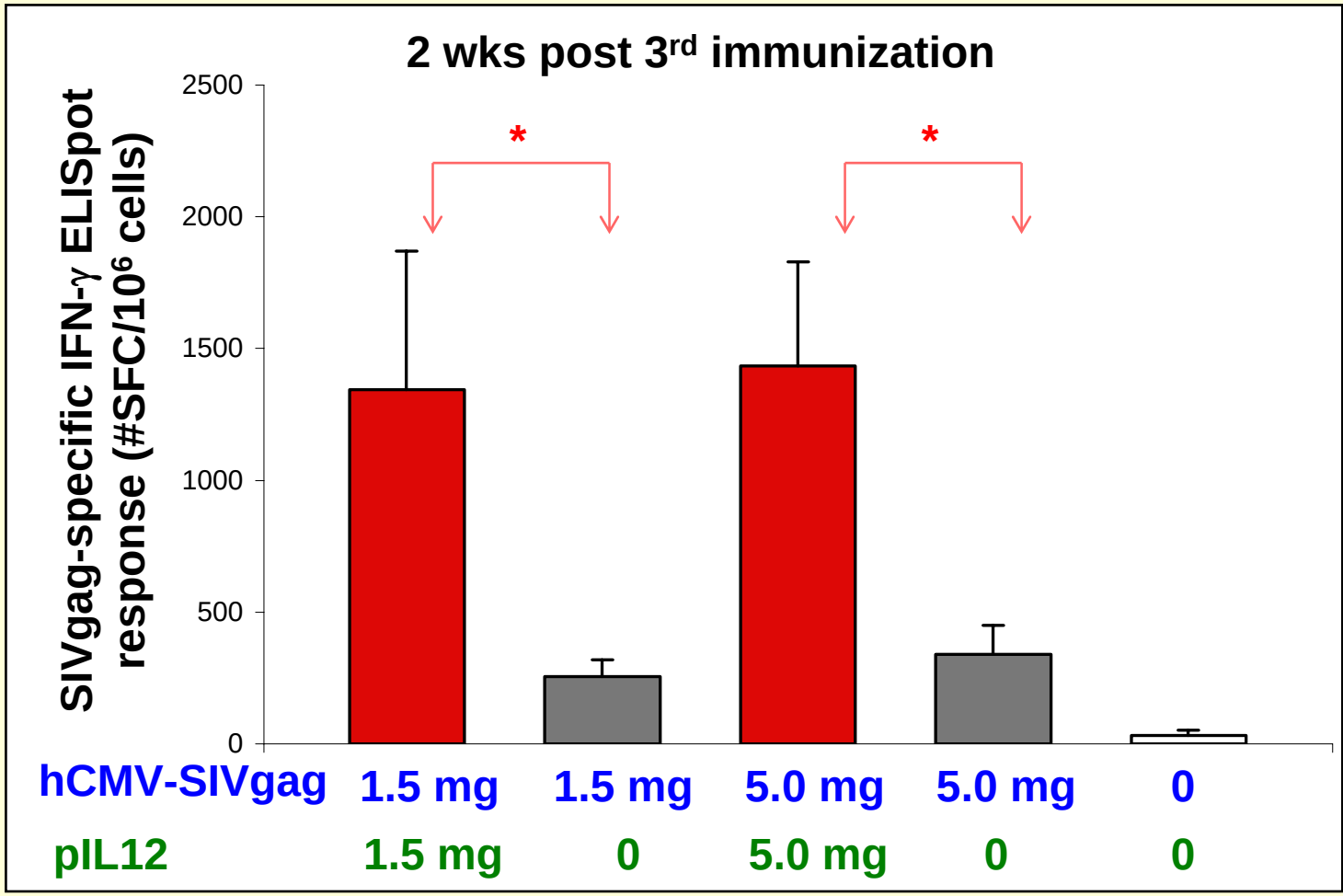
Dual promoter Rhesus IL-12



Wyeth Research

Plasmid-encoded rhesus IL-12 improves SIVgag-specific CMI responses in immunized macaques

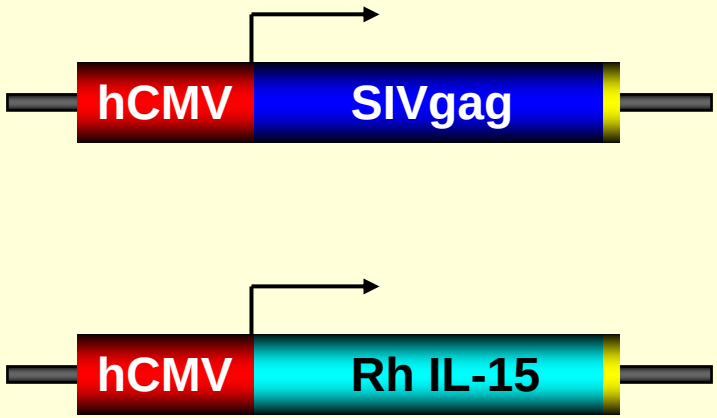




Schadeck EB, Sidhu M, Egan MA, et al., J. Immunol. submitted

* Statistically significant difference

IL-15

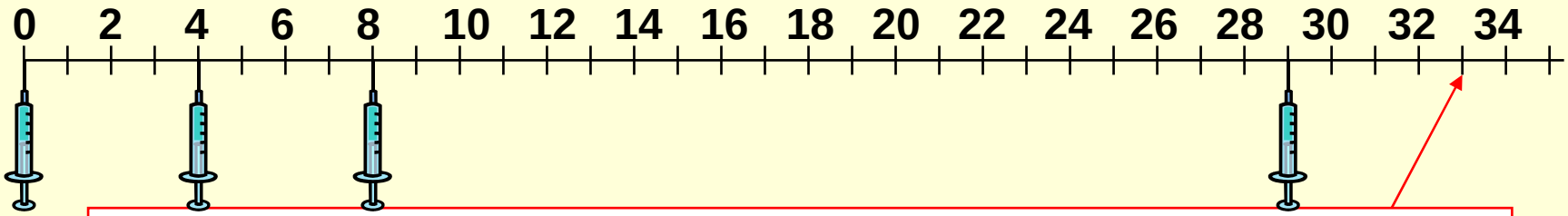


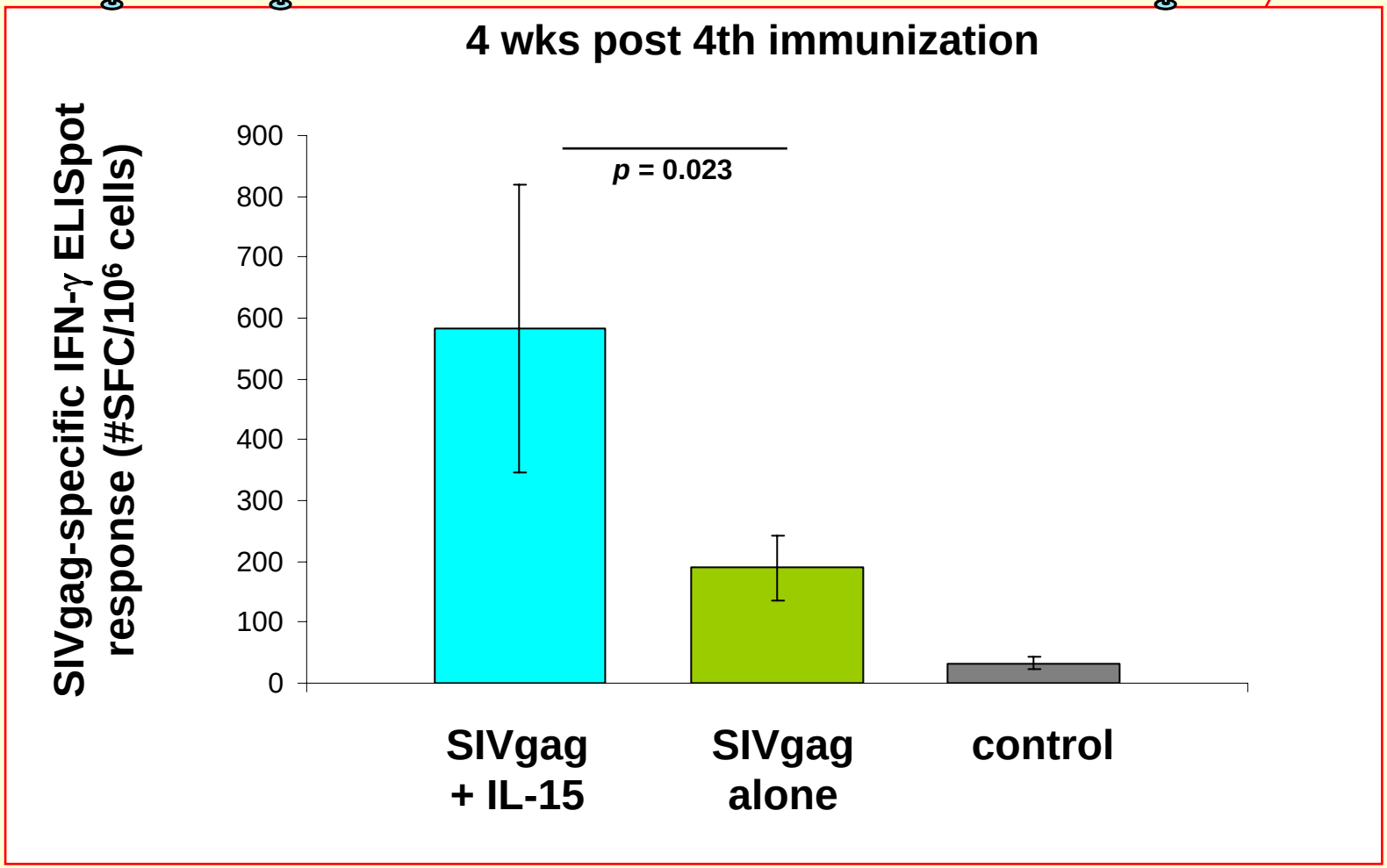
IL-15

- 15 kD glycoprotein
- Macrophages and monocytes are major producers
- Role in survival and expansion of naïve and memory CD8 T cells
- Regulator of NK-cell development and activity

Wyeth Research

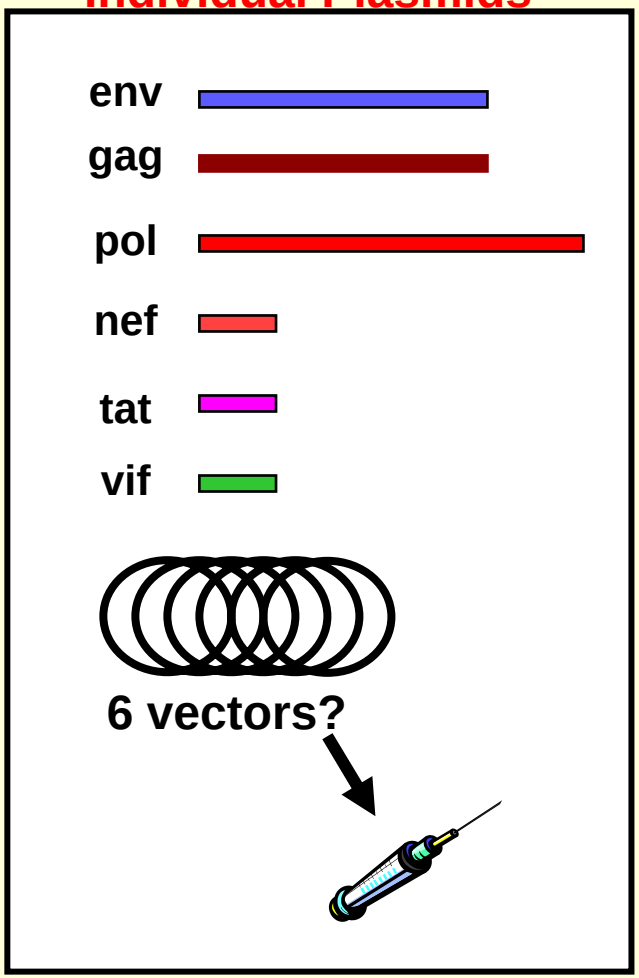
Plasmid-encoded rhesus IL-15 improves SIVgag-specific CMI responses in immunized macaques



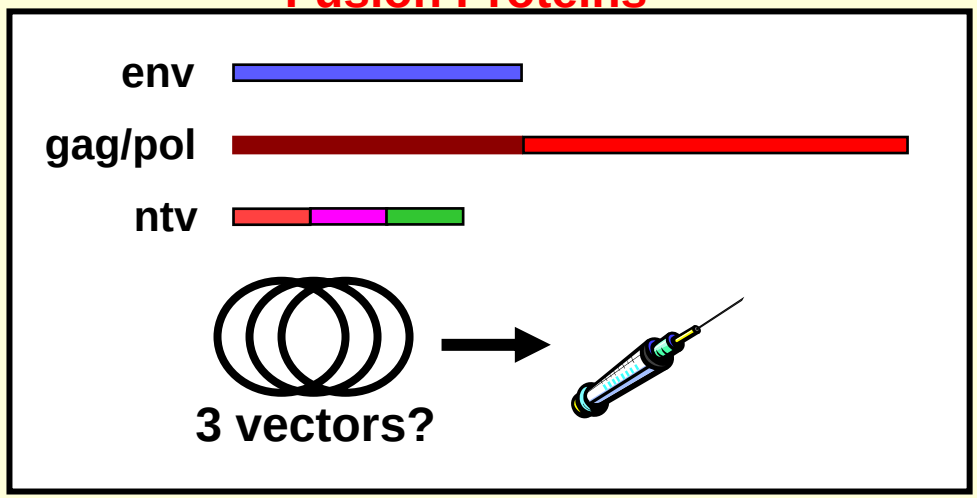


Optimizing pDNA vaccines encoding multiple viral antigens

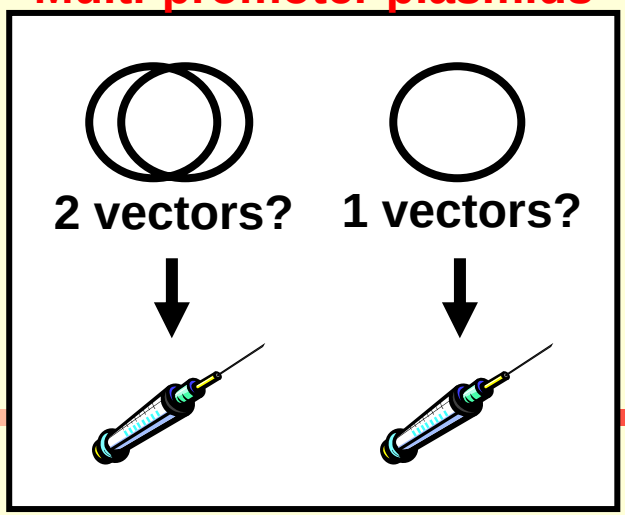
Individual Plasmids



Fusion Proteins



Multi-promoter plasmids

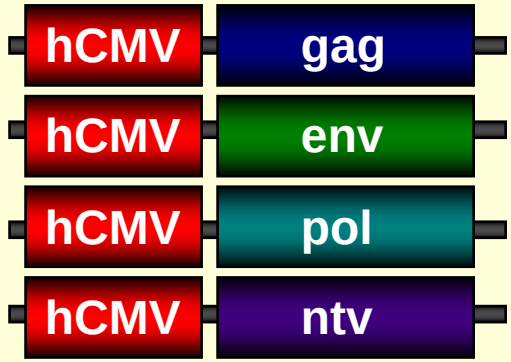


Wyeth Research

Summarized Results from Initial Round of Optimization

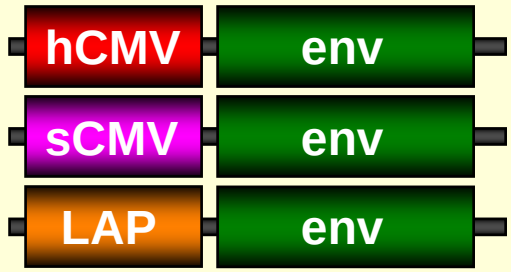
Relative immunogenicity of target HIV antigens determined

- Compared gag, env, ntv, pol
- Assessed individually
- Expression controlled by hCMV promoter/enhancer
- Env>Gag>Pol>NTV



Examined effect of promoter strength on relative immunogenicity

- hCMV>sCMV>LAP



Results used to design alternative plasmid vectors to deliver multiple antigens

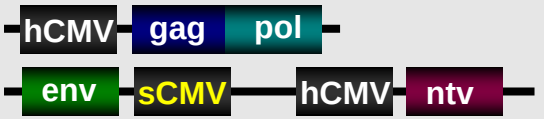
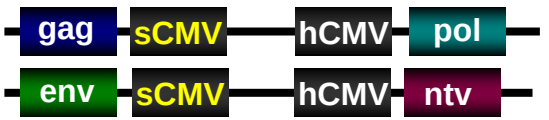
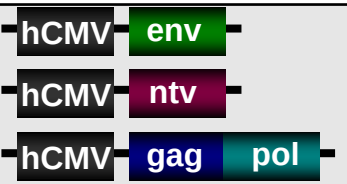
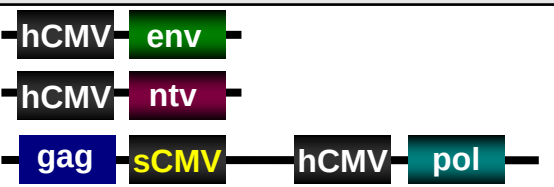
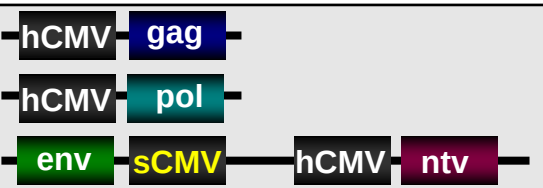
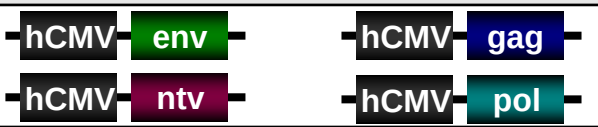
- 1, 2, 3, and 4 plasmid vaccine formulations tested

Wyeth Research

Analysis of vaccines formulated with 1-4 pDNAs

All vaccine formulations encode

- gag
- env
- pol
- ntv

pDNAS	Group	Dose per pDNA (μg)
	1a	100
	2b	50 each
	2c	50 each
	2d	50 each
	2e	50 each
	3a	33 each
	3b	33 each
	3c	33 each
	4a	25 each

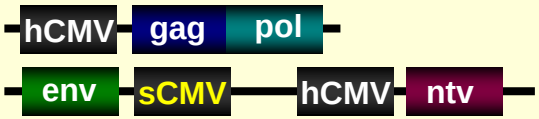
Mouse immunogenicity

Results

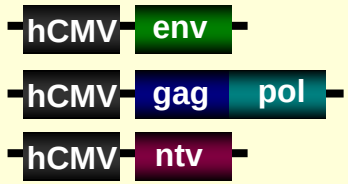
- Large, multi-promoter vectors performed less well
- Several pDNA vaccine designs were sufficiently immunogenic for further testing in non-human primates:

Most Immunogenic Compositions

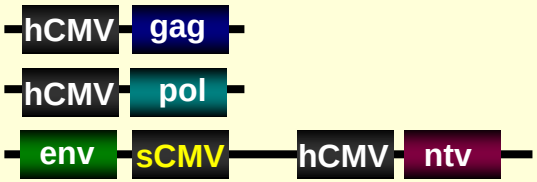
2d



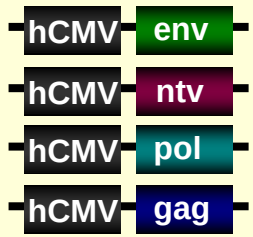
3a



3c



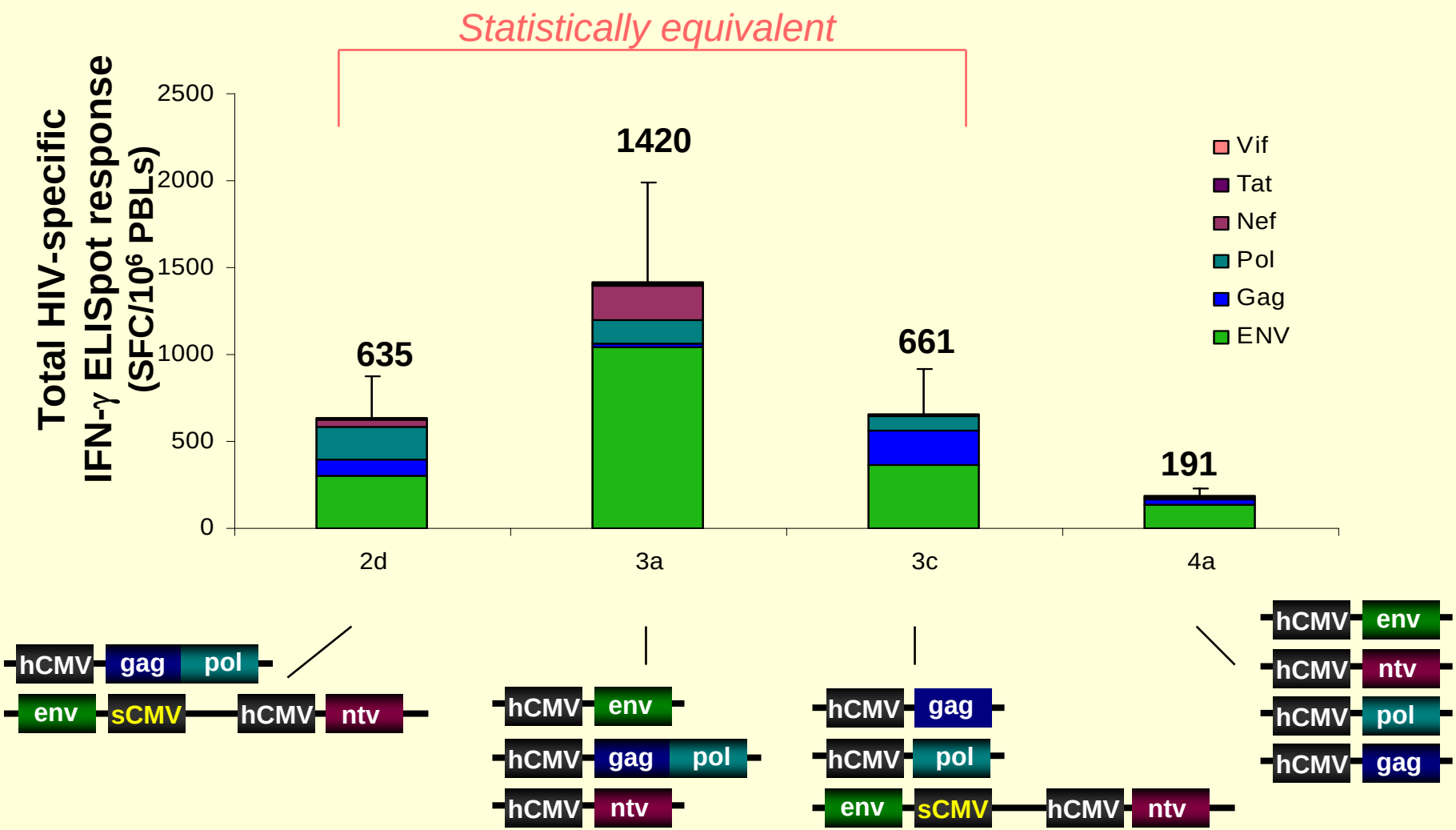
4a



Wyeth Research

Macaque Study Results

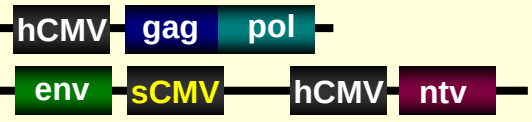
Total HIV-specific ELISpot PEAK Response 2 weeks post 3rd immunization



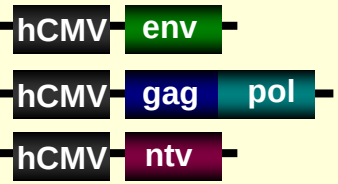
All include rhIL-12

Wyeth Research

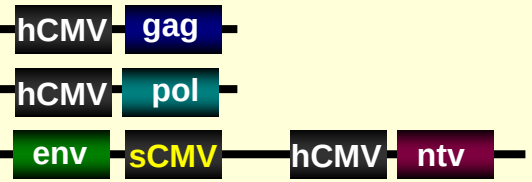
Macaque Study Summary



2d



3a



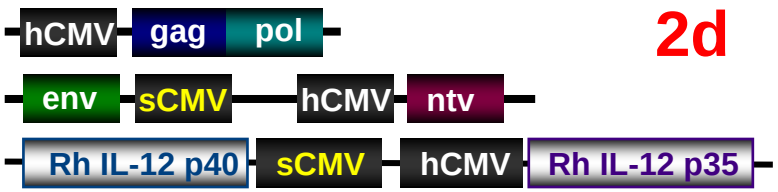
3c

Responses produced by 2a, 3a, and 3c were statistically equivalent

2d was selected for clinical trial

- Produced the most balanced immune response
- The simplest design - 2 plasmids + molecular adjuvant

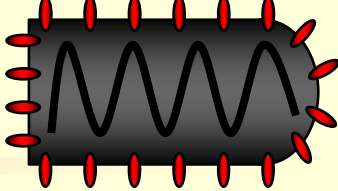
Clinical Trial Material

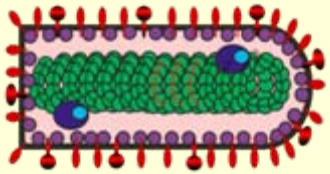


2d

Wyeth Research

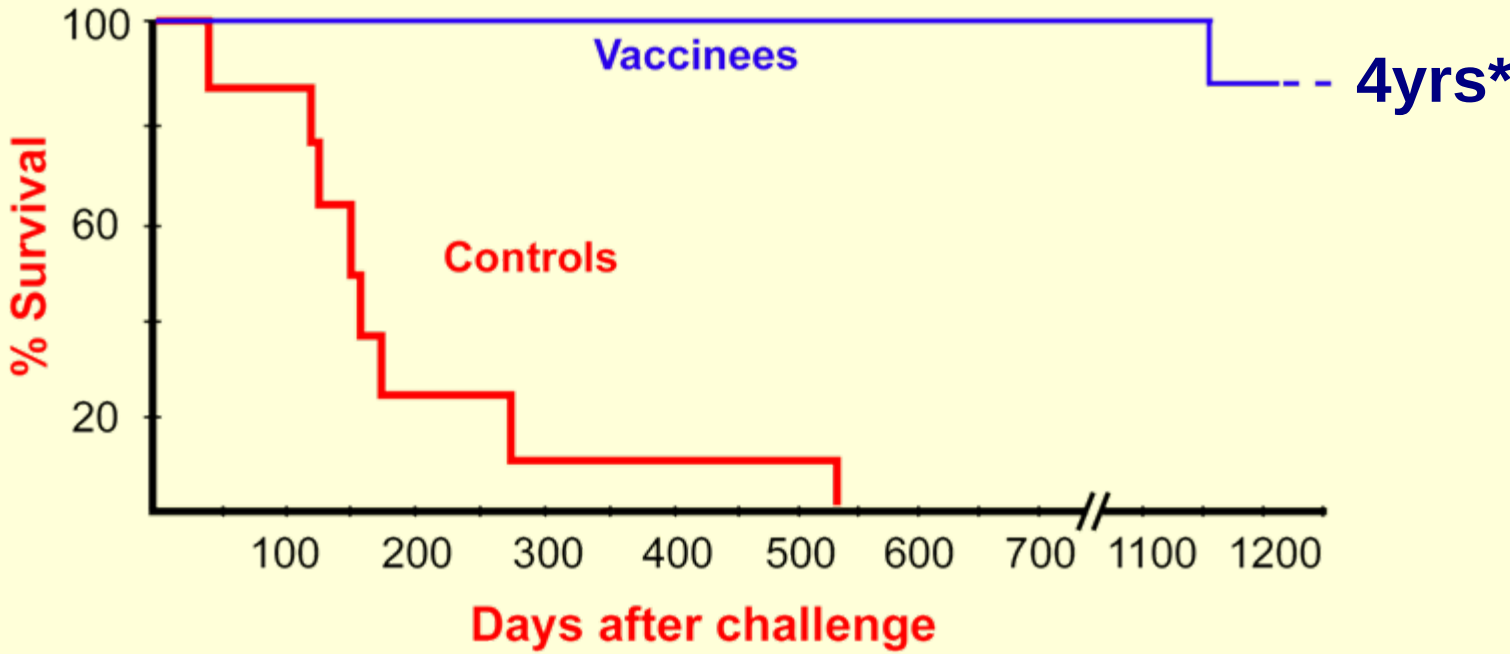
Significant Protection from SHIV Challenge Induced by Vaccination with rVSV Vectors





Immunize

Challenge



Rose, Marx, Luckay, Nixon, Moretto, Donahoe, Montefiori, Roberts, Buonocore, Rose . Cell 106:539-49, 2001

***A second animal developed gluten enteropathy and was euthanized.**

- **Normal CD4 counts**
- **Undetectable virus load**

VSV-HIV Vectors
Summary of Preclinical Experience

Preclinical efficacy demonstrated in Macaque SHIV challenge model

Desirable cellular immune responses resulted from VSV-gag / VSV-env vaccination

No adverse events caused by rVSV-HIV vaccination

NIH Contract
Wyeth, Yale, Tulane

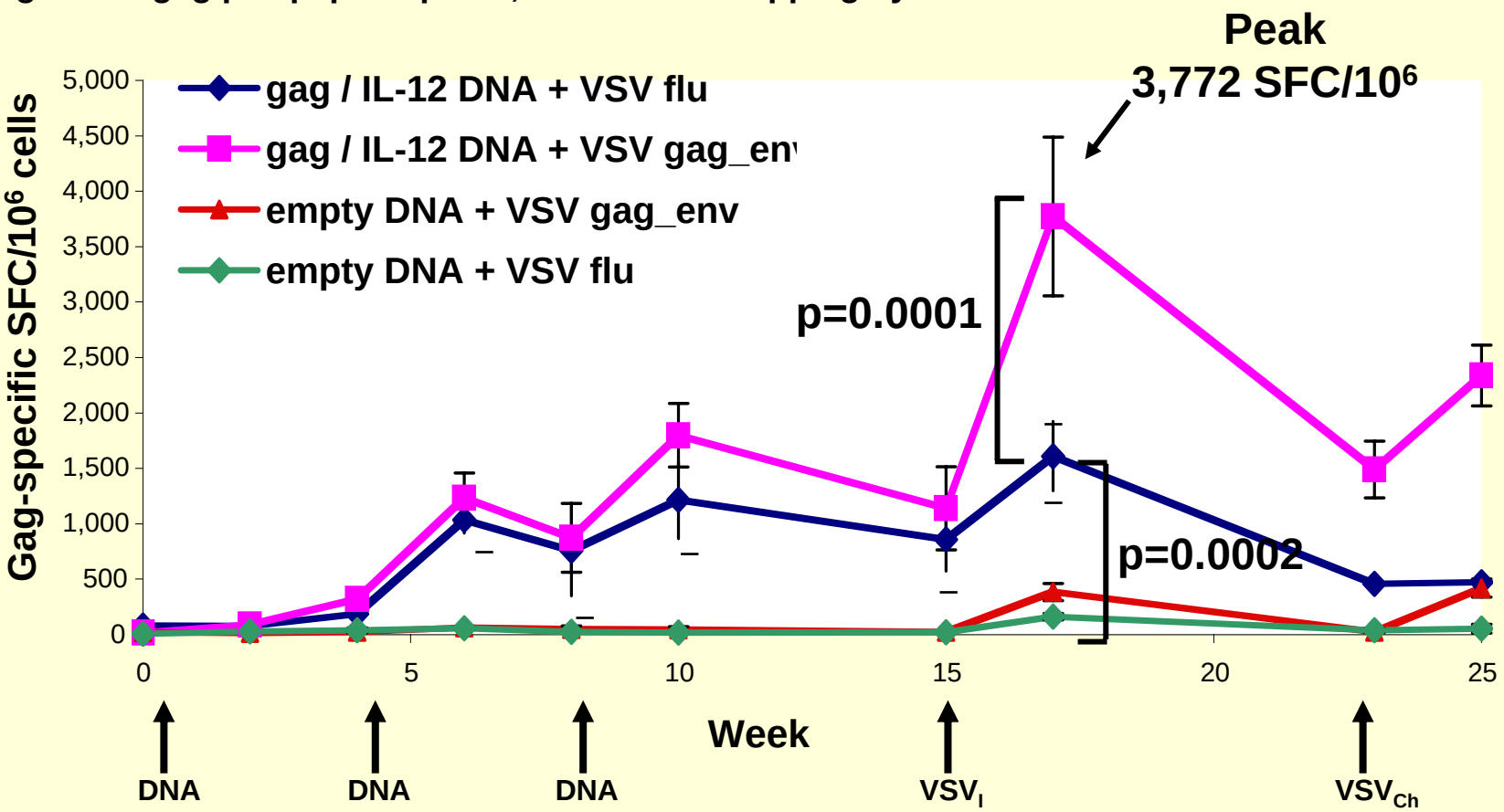
Wyeth Research

Prime-Boost Potential

pDNA prime/ rVSV boost - ELISPOT

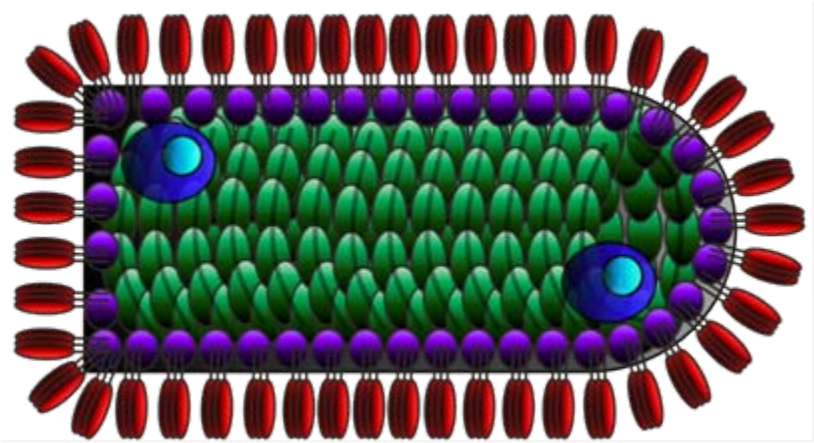
SIVgag-specific INF-γ ELISPOT Responses

Antigen: SIVgag p55 peptide pools, 15 mers overlapping by 11aa



VSV-HIV Vaccines for use in Humans

Eliminating Risk

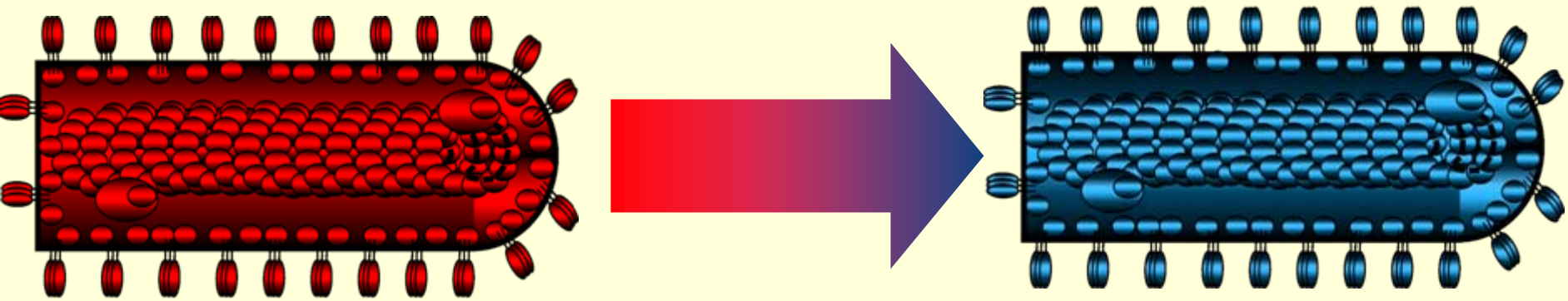


- **Wild-type VSV is known to be neuroinvasive/ neurovirulent in young rodents**

- **NV potential must be addressed before commencing with human clinical studies**

Wyeth Research

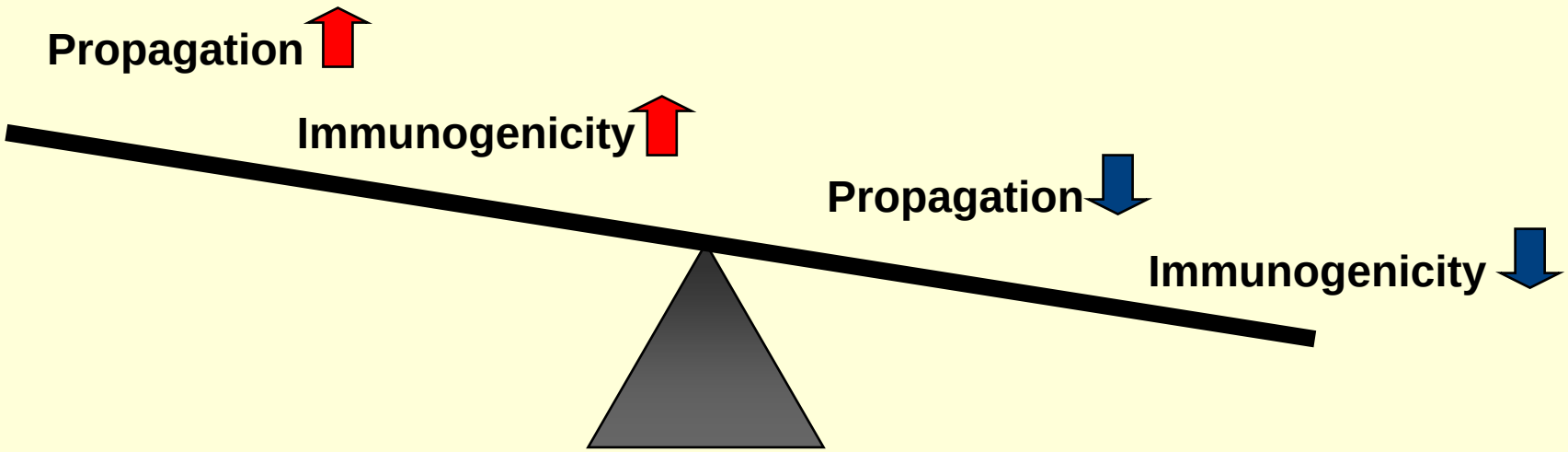
Balancing Safety and Immunogenicity



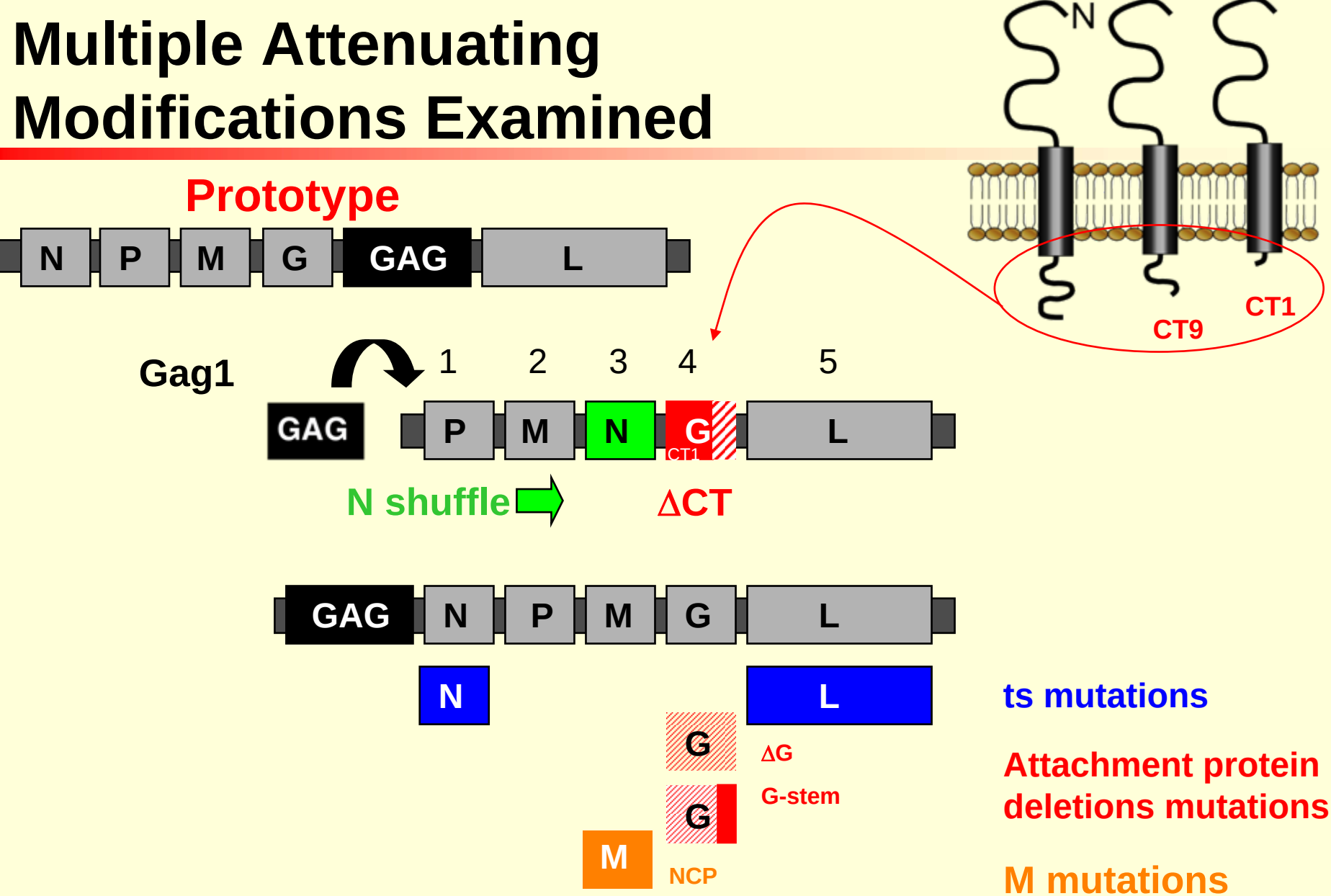
Prototype

Attenuated

Low N.V. potential



Multiple Attenuating Modifications Examined



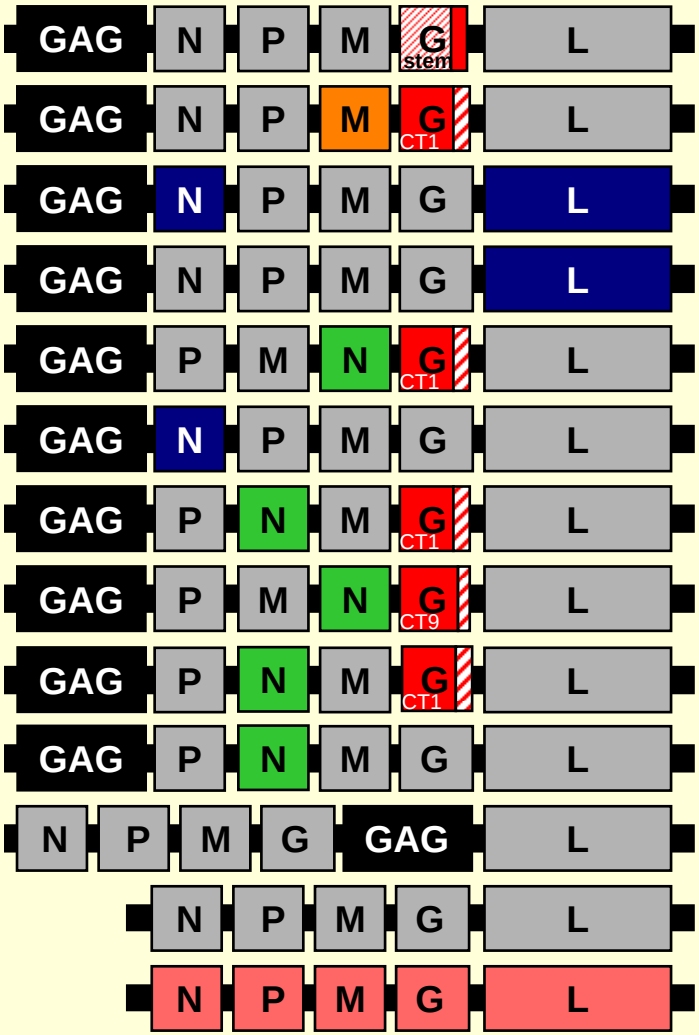
ts mutations

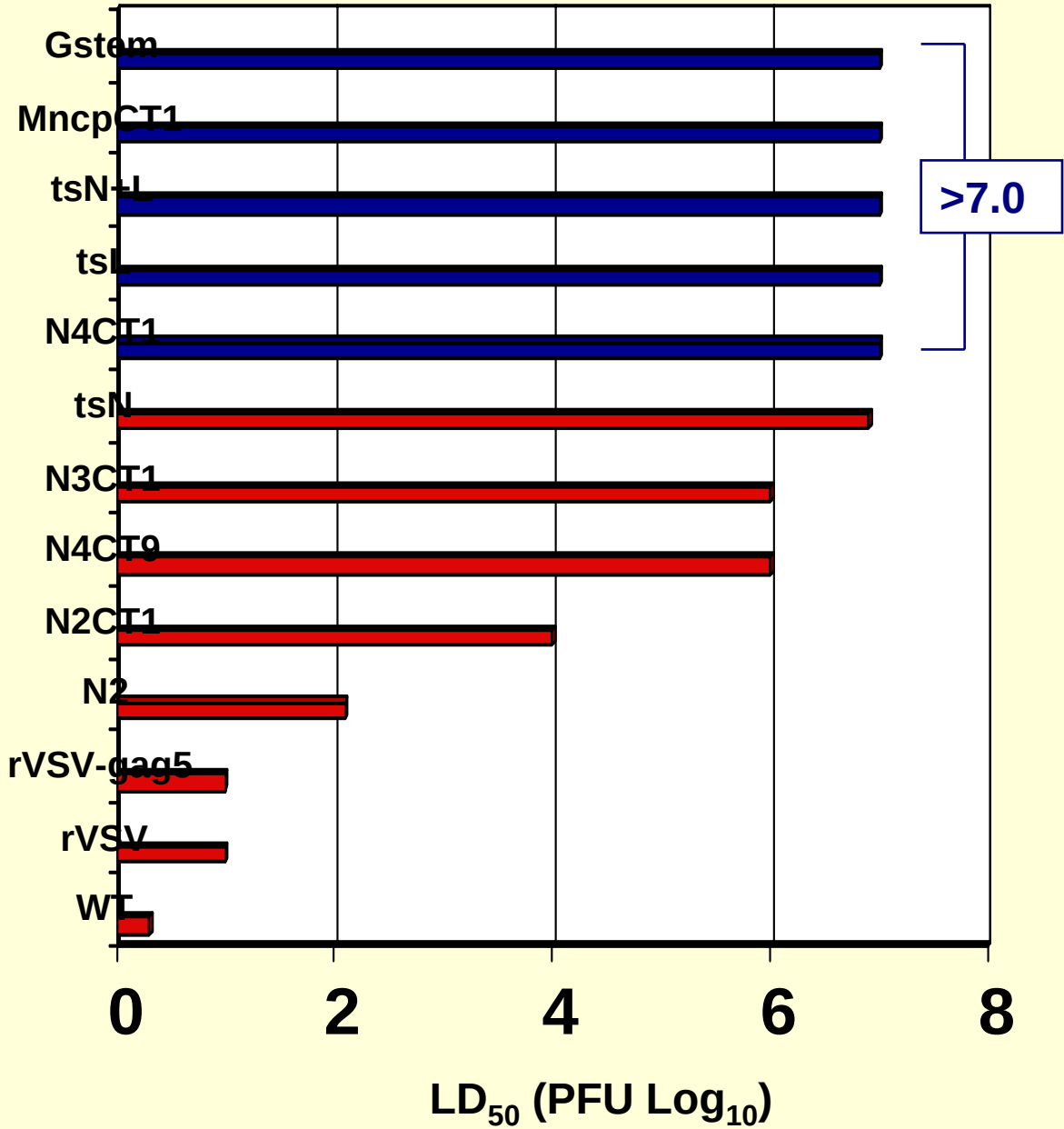
Attachment protein deletions mutations

M mutations

Roberts, A., L. Buonocore, R. Price, J. Forman, and J. K. Rose. 1999. J Virol 73:3723-32
Wertz, G. W., V. P. Perepelitsa, and L. A. Ball. 1998. Proc. Natl. Acad. Sci. USA 95:3501-3506.
Jayakar, H. R., and M. A. Whitt. 2002. J Virol 76:8011-8.
Pringle, C. R. 1970. J Virol 5:559-67.
Robison, C. S., and M. A. Whitt. 2000. J Virol 74:2239-46.

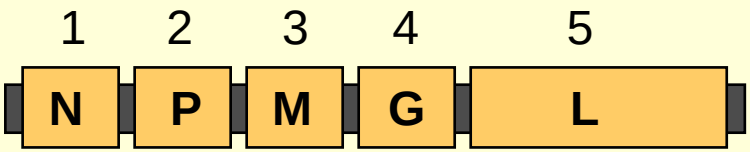
Evaluating Attenuated Vectors - Mouse I.C. LD_{50}



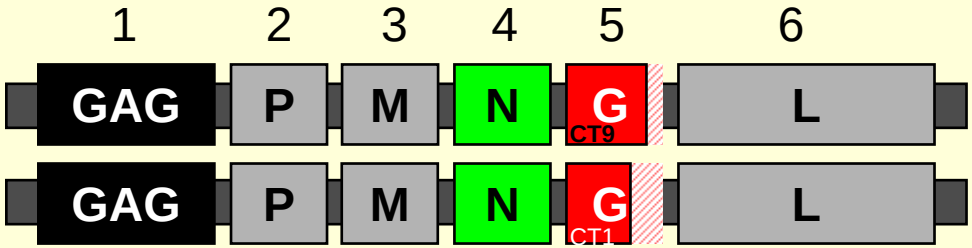


Menu of Attenuated Vectors

Highly Attenuated / Low NV potential



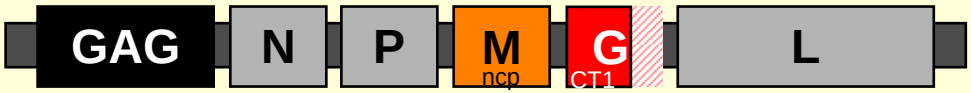
WT



GAG1-N4CT9

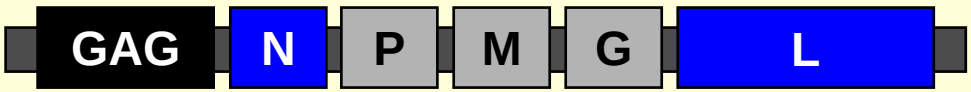
GAG1-N4CT1

N gene shuffle & ΔCT



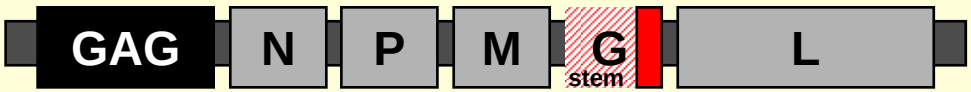
GAG1-MncpCT1

M mutations & ΔCT



GAG1-tsN+L

ts mutations



GAG1-Gstem

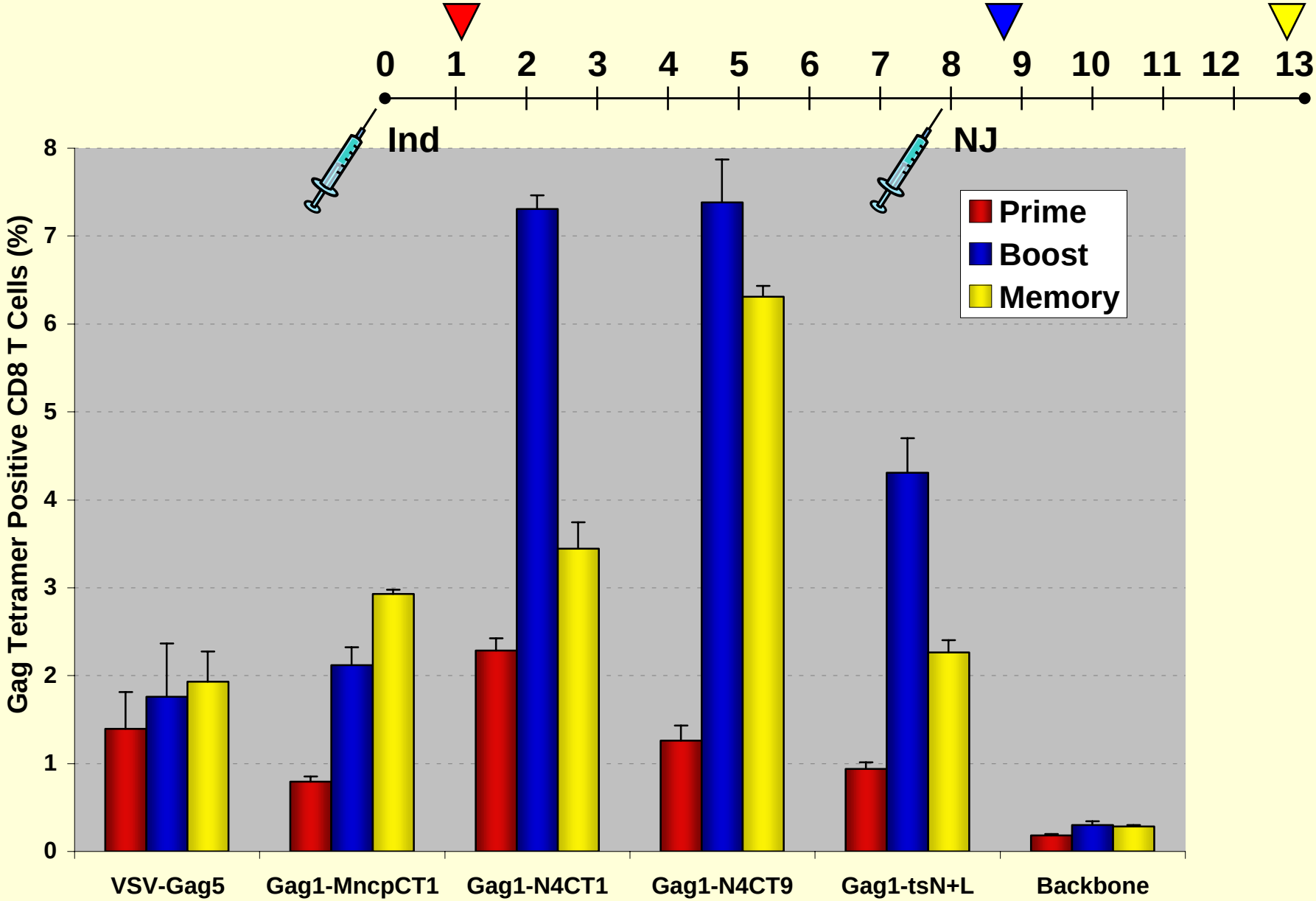
Nonpropagating

Δ attachment protein

Wyeth Research

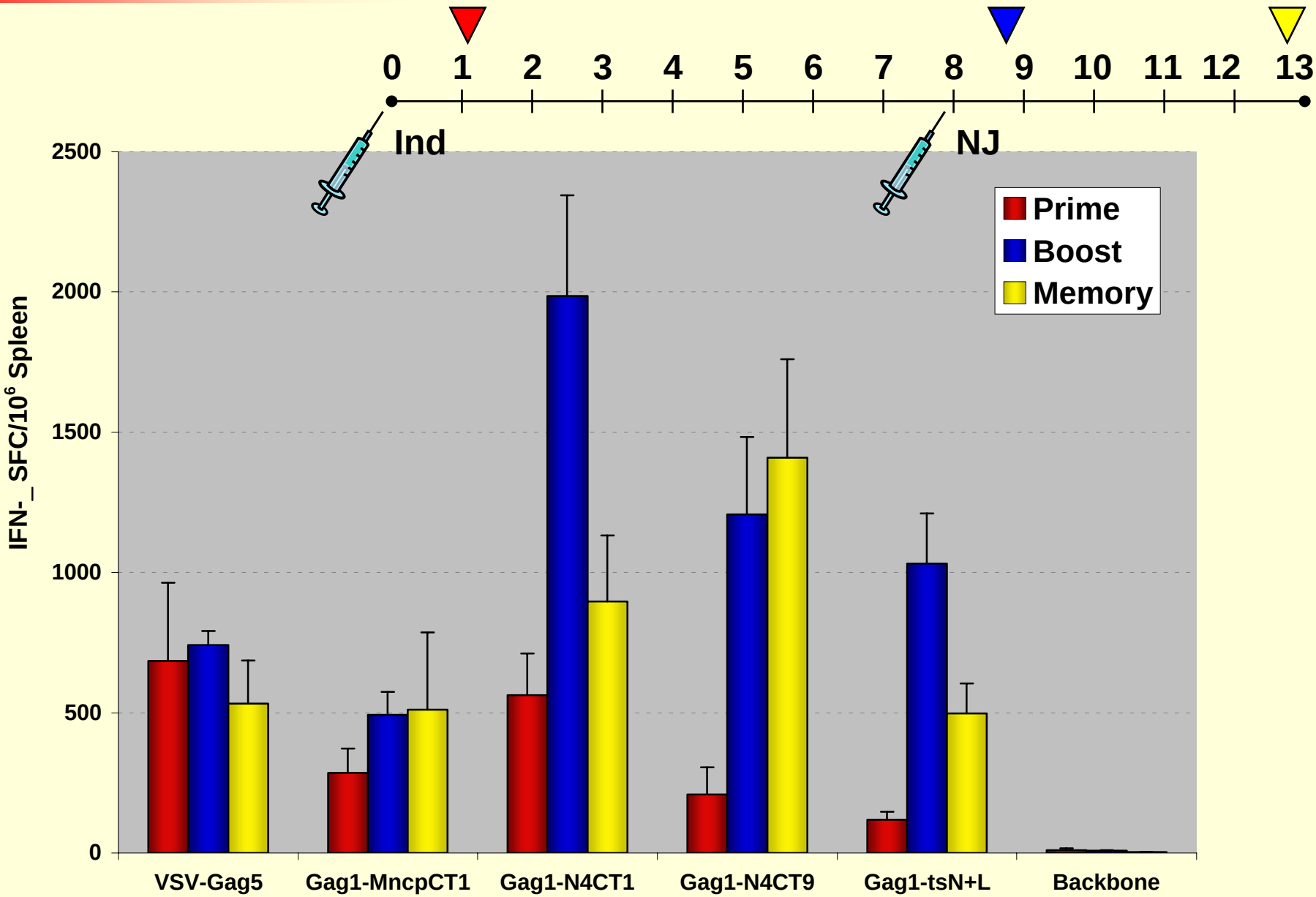
IMMUNOGENICITY - GAG Tetramer Staining (Mouse IM)

Replication-competent attenuated vectors



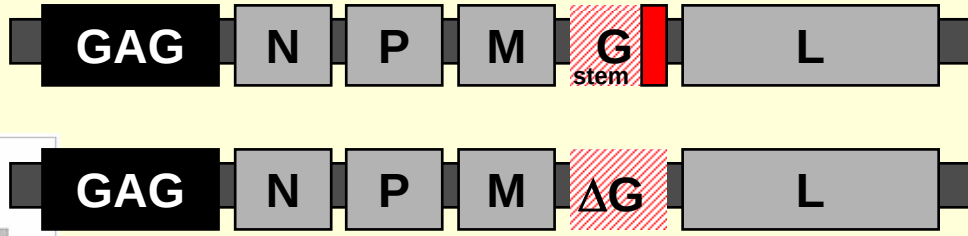
IMMUNOGENICITY - Gag IFN-γ ELISPOT (Mouse IM)

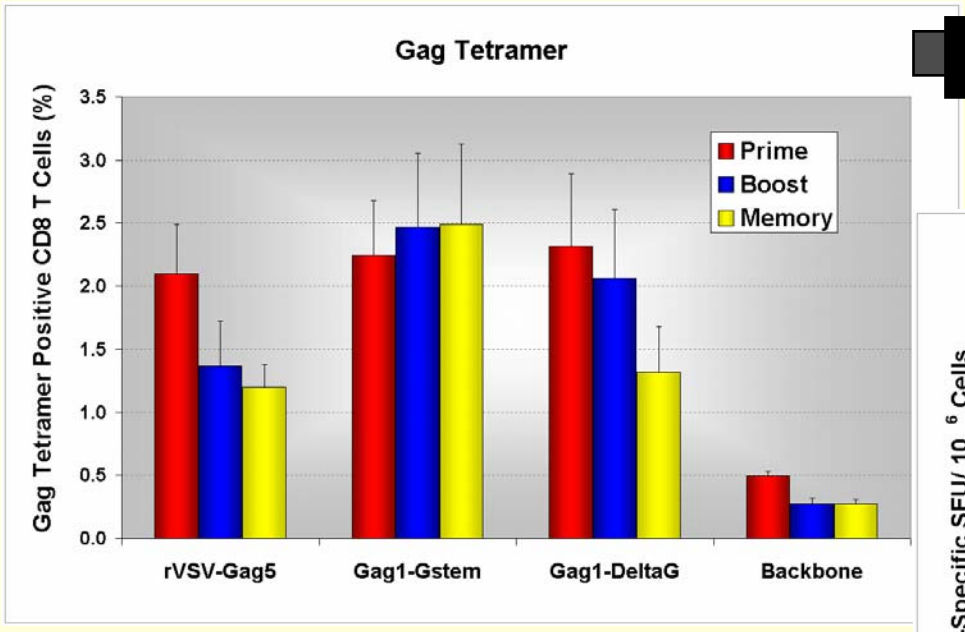
Replication-competent attenuated vectors

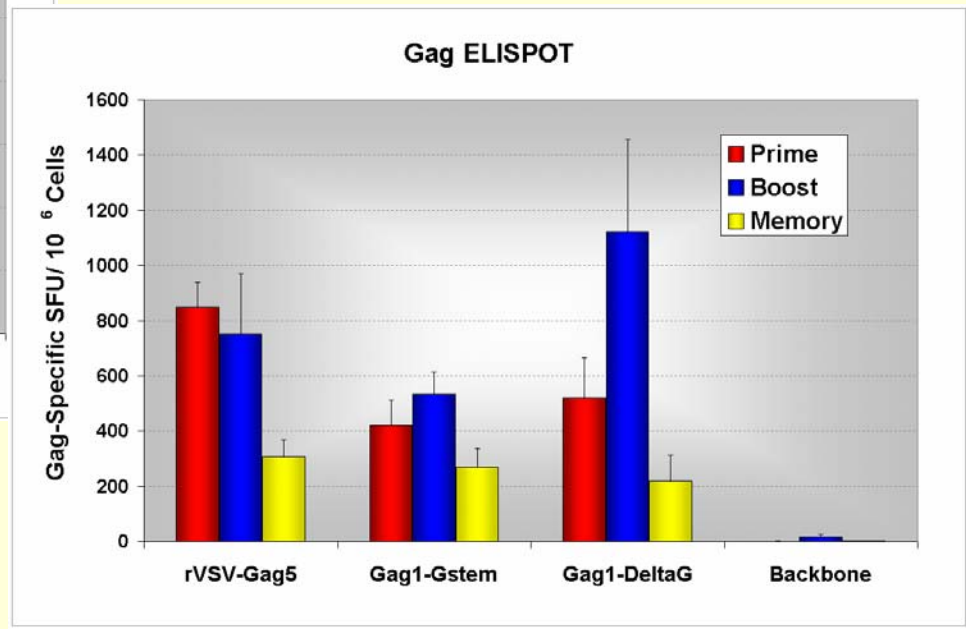


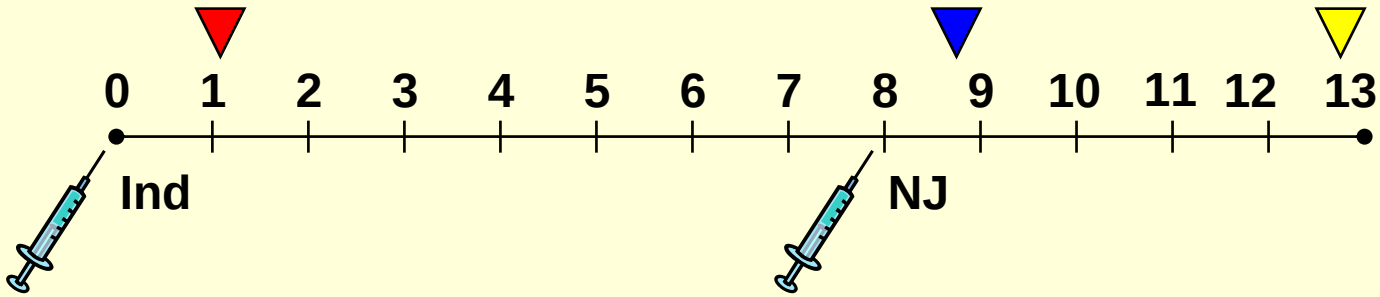
Anti-Gag p24 IgG Serum Titers (Mouse IM)

Nonpropagating vectors

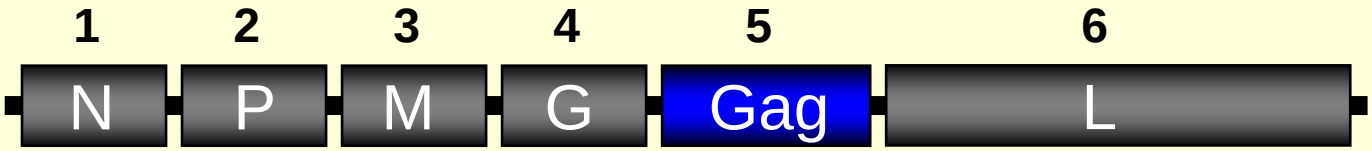






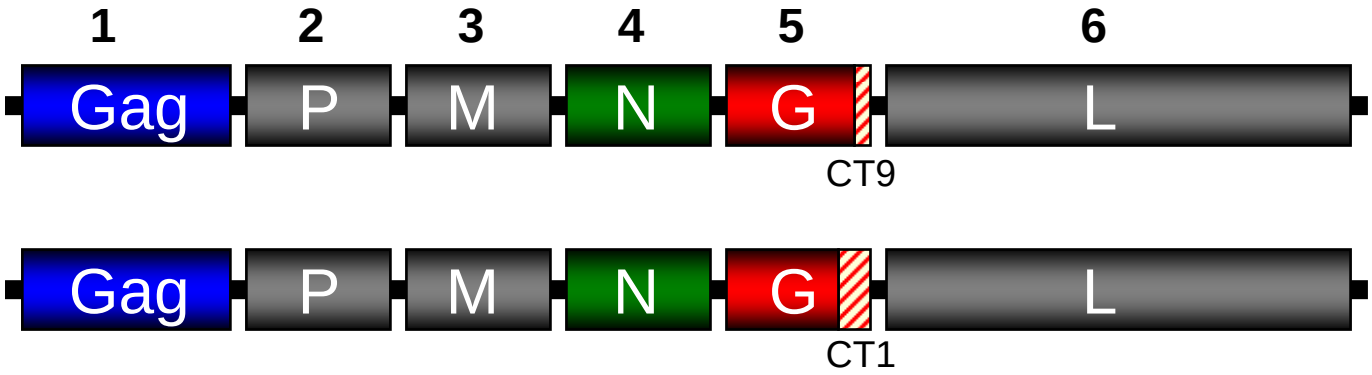


Top Candidate Vectors



Rose prototype

- Replication competent
- Immunogenic
- Minimal NV in mice and macaques
- Immunogenicity: N4CT9>N4CT1
- Both propagate in Vero cells

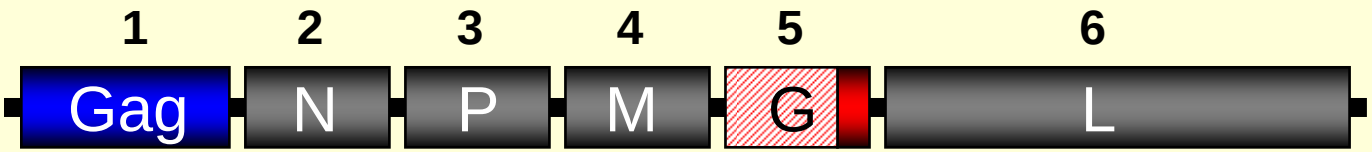


Gag1-N4CT9

More attenuated

Gag1-N4CT1

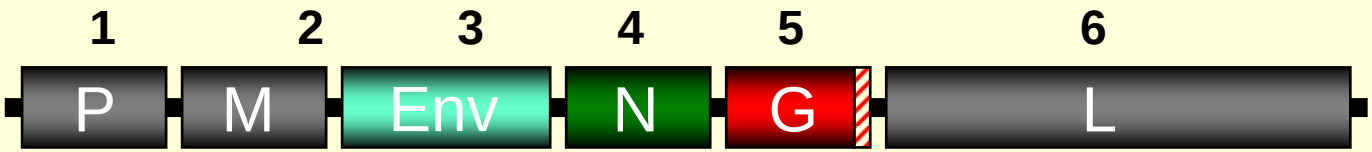
- Immunogenic
- A 'Replicon' vector
- High degree of safety
- Difficult to prepare in large quantities
- Manufacturing methods under development



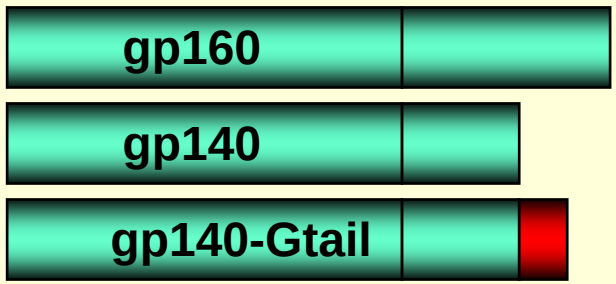
Gag1-Gstem

Continued Research & Development

- Env immunogenicity

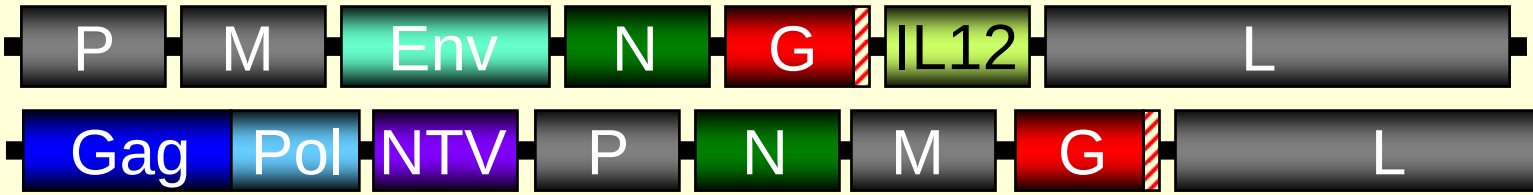


Env3-N4CT9



Glycoprotein expression from Position 1 is toxic

- Expand repertoire of antigens
- Immune modulators



- Continue development of a scalable VSV replicon vector

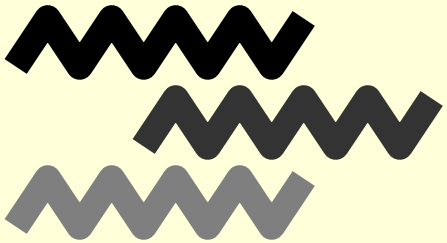
GAG-N4CT9

Clinical Trial Preparation

- Scale up…….. √
- Purification…….. √
- Formulation……. In progress
- Assay development……. √
- IND……. Package in preparation
- Manufacture & Fill……. Scheduled

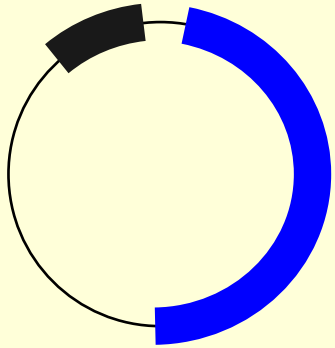
Wyeth Research

Wyeth Vaccines HIV Program



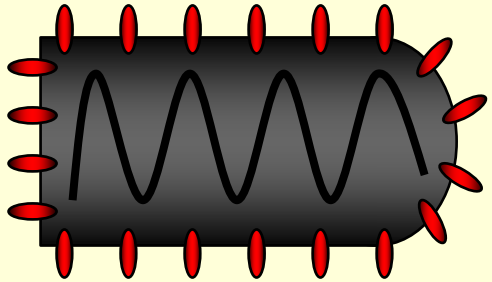
Th-CTL Peptides

In Phase I



Plasmid DNA

In Phase I



Viral Vector

Preclinical

Wyeth Research

Potency Definition

- **ICH Q6B section 2.1.2 Biological Activity**
  - Potency (expressed in units) is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties, whereas, quantity (expressed in mass) is a physicochemical measure of protein content. Mimicking the biological activity in the clinical situation is not always necessary. A correlation between the expected clinical response and the activity in the biological assay should be established in pharmacodynamic or clinical studies.
- **Potency can describe the immunological response to an antigen in the target host (Phase 3), animal model (Phase 1/2), or a quantitation of the antigen (Phase 1)**
  - Animal testing provides some prediction of activity in humans, although this is difficult with antigens that specifically target human epitopes
  - Potency testing must evolve along with the information and experience gained from clinical trials where in vitro and animal testing can be correlated with actual results in humans
  - Therefore, in Phase 1, most “potency” testing is a quantitative or even qualitative in vitro measurement of antigen concentration and expression

Experimental HIV Vaccine Potency

An idealized definition might be:

Composition that elicits immunological responses correlating with protection in greater than ??% of vaccinees

HIV vaccines challenges:

- No certain correlates of protection
- Numerous novel and evolving vaccine delivery modalities
- Many antigens and complex vaccine formulations under consideration
- Poorly immunogenic antigens
- New and complex assays used to quantify immunogenicity
- Immune compromised subjects

Defining Potency before Correlates of Protection are Established

Reproducible ('Validatable') and practical measure of potency that ensures consistent delivery of a defined quantity of antigen or vector

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Wyeth HIV Vaccines

- **Three approaches**
  - DNA plasmid
  - CTL Peptide
  - VSV Vector
- **All in development or early Phase 1 stage**
- **No clear correlate of protection**
  - But focusing on CTL response
- **Plans for Phase 3**
  - Correlate in vitro with in vivo results
    - Animal immunogenicity or clinical results

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Vaccines – Potency Assay Challenges

- **Multiple novel platform approaches**
- **Multiple molecular/immunological targets**
  - May require a multi-assay approach during early phase development
- **Animal models are only an approximation**
- **Despite scientific rigor and innovations, the final Potency Assay must be "validateable"**
- **Correlation of structure and function**

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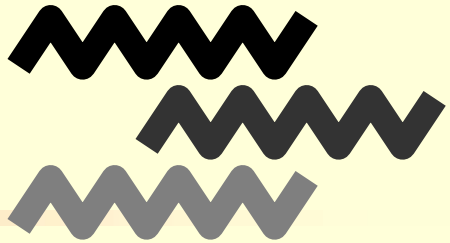
Th-CTL Peptide Potency



Short peptides (less than 50 residues)

Similar to a small molecule pharmaceutical

Potency based on peptide sequence and unit mass per formulated dose

- Sequence determined for peptides used in formulation
- Identity and quantity of peptides determined by HPLC in vaccine formulation

Limitations

Assays do not measure biological or immunological activity

- Uptake, processing, and presentation by antigen-presenting cells
- Quality of induced immune response (breadth, magnitude)

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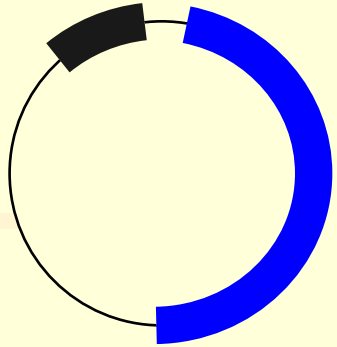
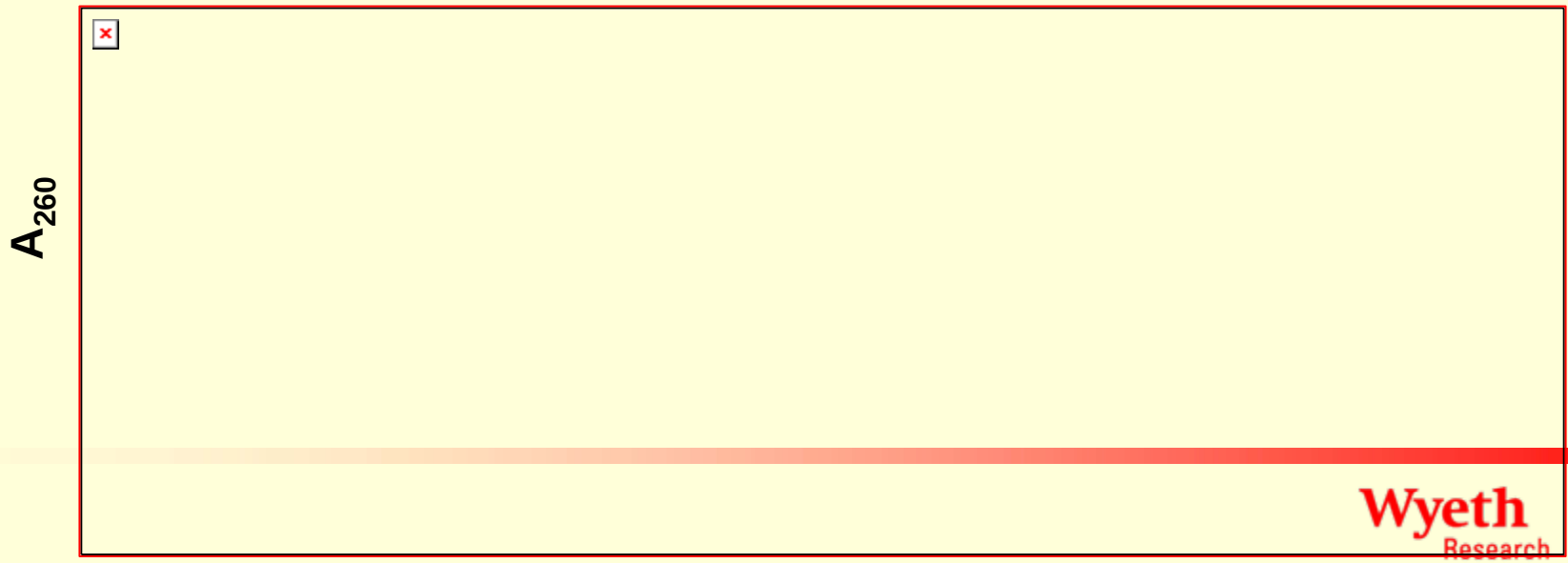
Plasmid DNA Vaccine Potency



Potency currently defined by

- Mass of DNA
- Percentage of supercoiled form determined by HPLC

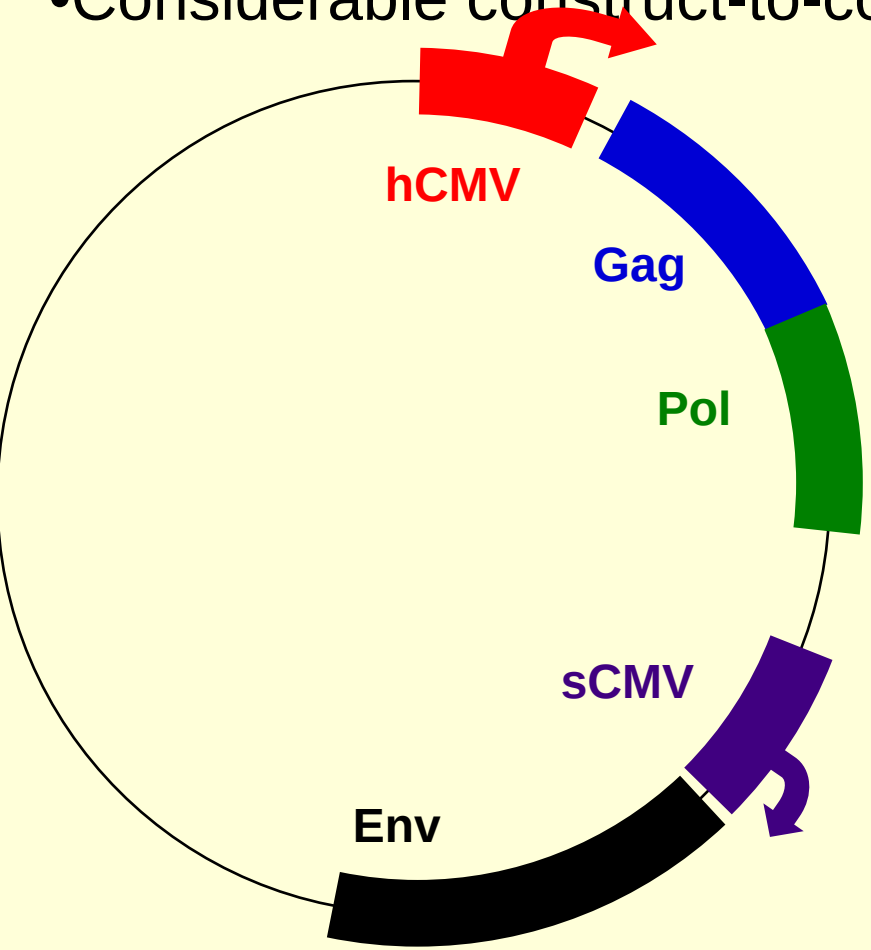
Identity determined by DNA sequence



Factors Affecting pDNA *Specific Activity*

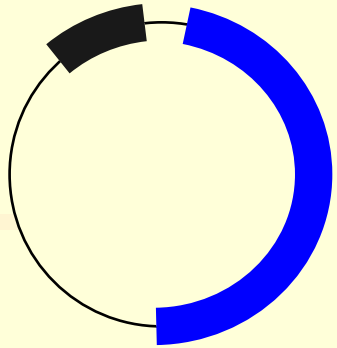
Limitations

- Does not measure biological activity or immune responses
- Considerable construct-to-construct variation expected



- Promoter Strength
- Processing pre-mRNAs
- Nuclear export
- mRNA stability
- Translation efficiency
- Antigen processing
- Antigen presentation

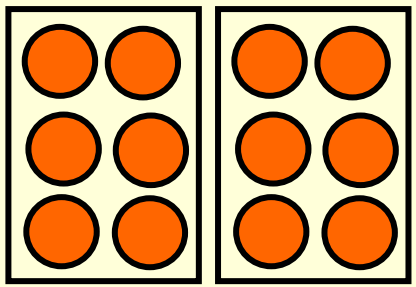
Verification of pDNA-encoded Antigen Expression *In Vitro*



Plasmid DNA

Transfection

3t3, RD, 293 and COS cells



48 hrs later

Harvest supernatants and cells

Analyze gene expression by ELISA and/or western blots

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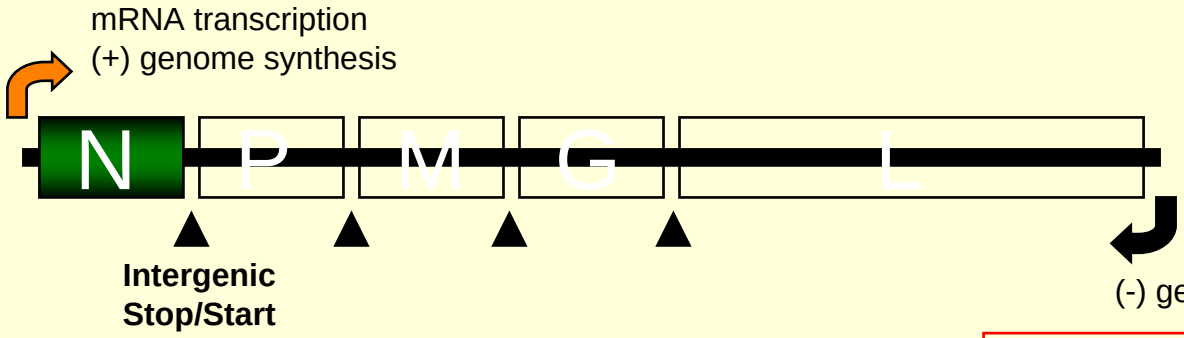
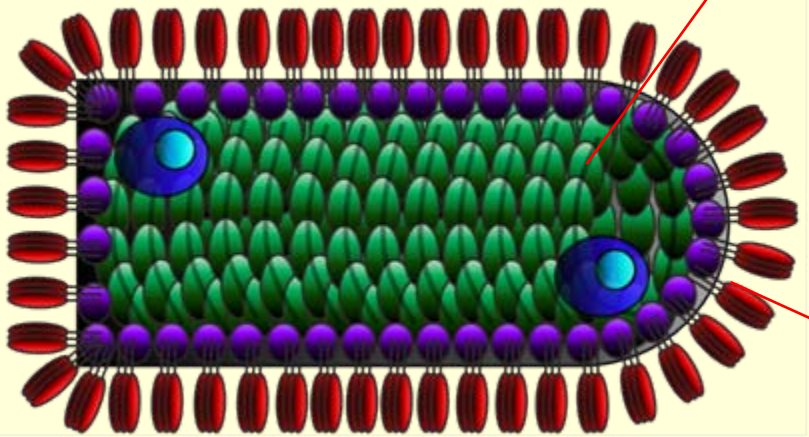
Vesicular Stomatitis Virus





RNA genome
- **Nonsegmented,**
- **Single-stranded**
- **Negative-sense**

Envelope

- Nucleocapsid
- Phosphoprotein
- Matrix protein
- G protein
- Large protein (RNA Pol)

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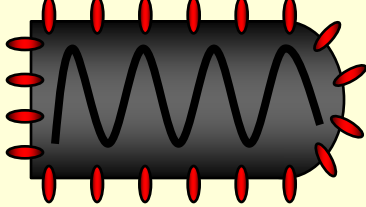
Factors VSV Vector Affecting Potency



- Efficient delivery - infection
- Stability - live agent
- Level of attenuation / replication competence
- Efficiency of transcription and translation

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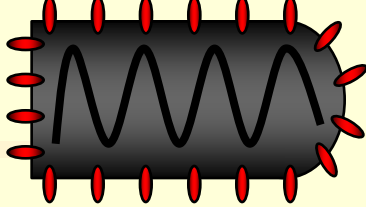
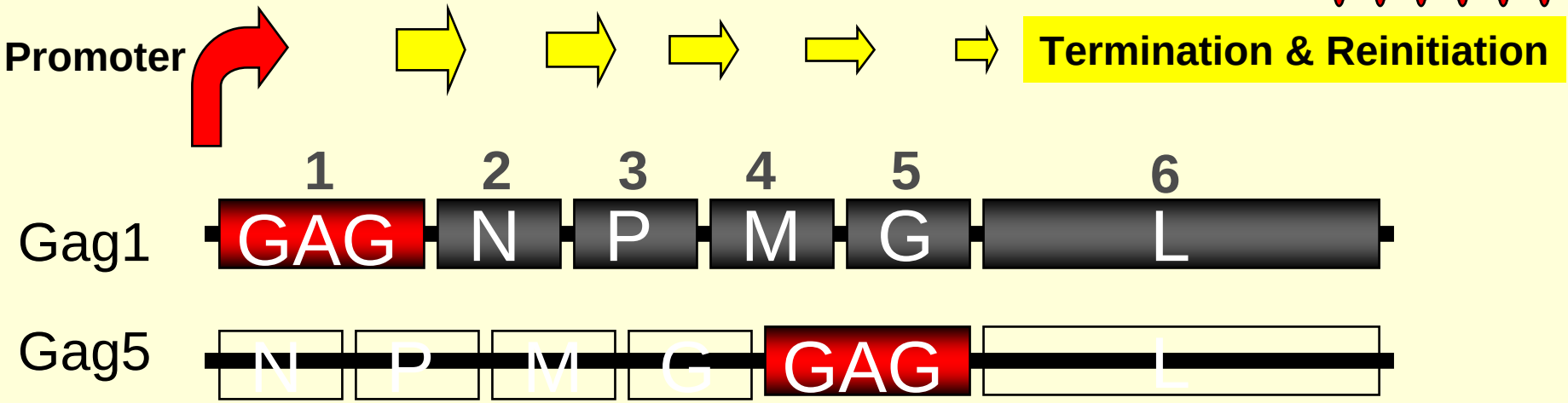
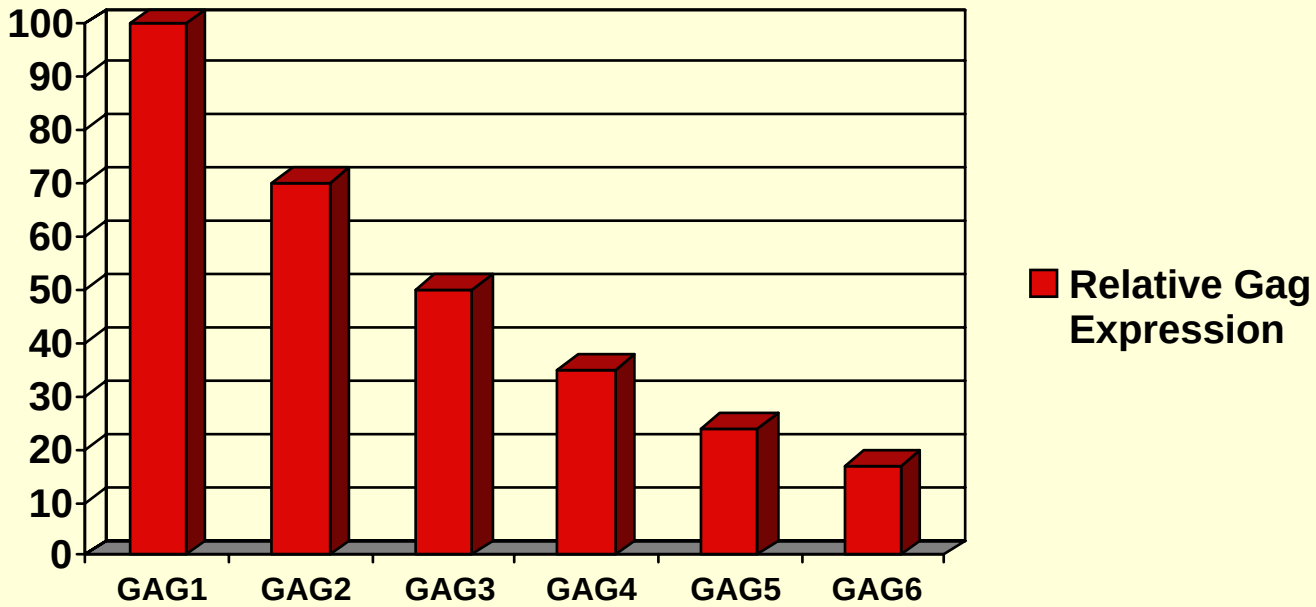
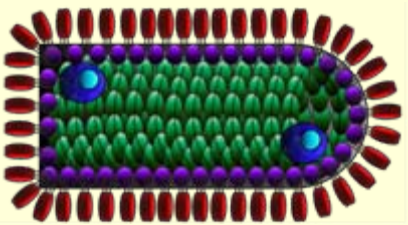
VSV Vector *Specific Activity*

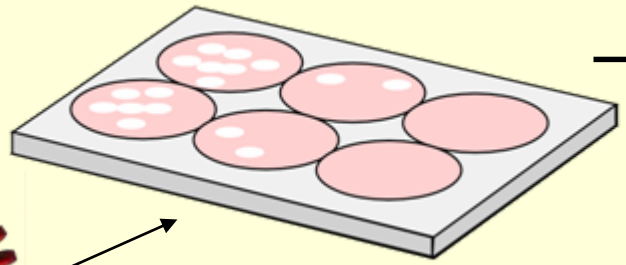




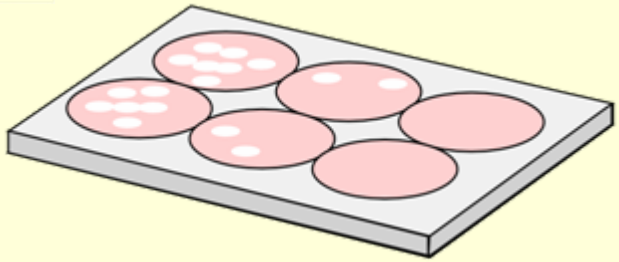


Verification of Antigen Expression

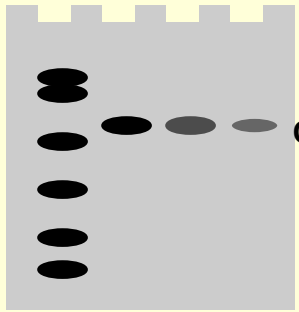




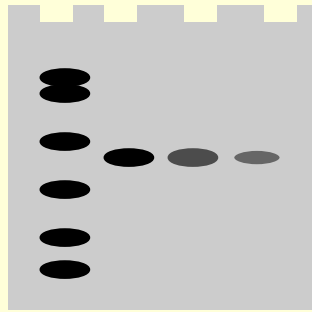
Plaque-Forming Units



Antigen Expression

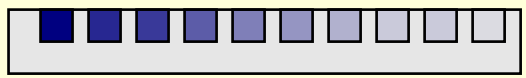


Gag



VSV N

Western or ELISA



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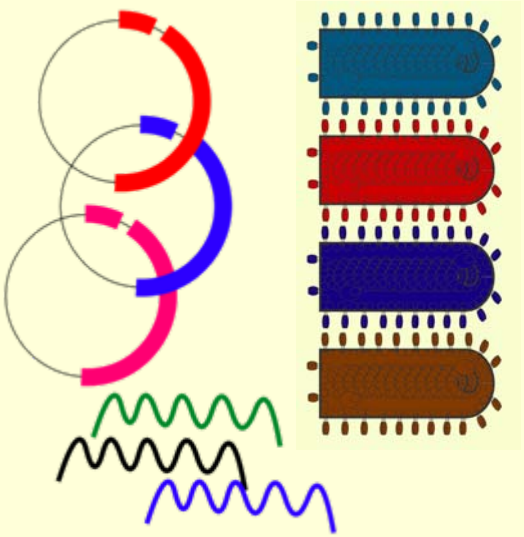
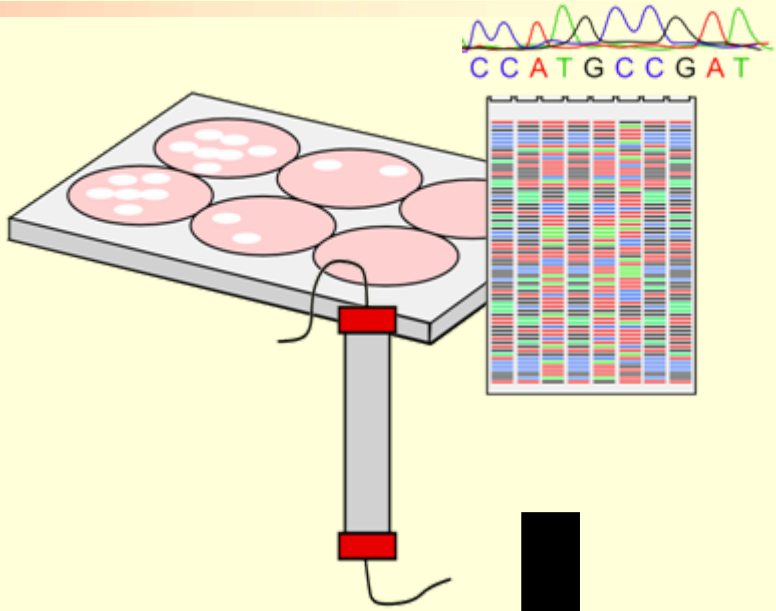
Potency Determination For Phase I



- Dose (mg, PFU)
- Purity
- Identity
- Antigen expression (pDNA and viral vectors)



Immunogenicity testing

Data comparison

Phase I

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Multiple Antigen Constructs (evolving assays)

- **Potency testing for each epitope or representative epitopes?**
- **We propose that only select antigens may need be targeted for measurement in the in vitro characterization assays**
  - Plasmid or vector - measure one antigen/promoter
  - Measure all peptides in mixture
- **Expression and sequence are measured during characterization of the drug substance**

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In Vitro and *In Vivo* Potency Correlation

- **Because our plasmids/peptides/vectors will Not change during production (no mutation, degradation, etc.) there is no need to test in vivo potency as a release test.**
- **Establishing that the vaccine is identical to a construct that has been proven to elicit a specific immune response should be adequate for release.**
- **Therefore we contend that vector replication titer (release) and in vitro expression (characterization) will correlate sufficiently with immunogenicity to use these two surrogates for vector vaccine potency.**

Prime-Boost

Each component needs to be released on its own based on criteria that identifies it as identical to a vaccine that has been shown to produce an acceptable immune response in animals (or humans) when given as part of the whole vaccine.

- e.g. 3 doses Prime, 2 doses Boost

Humoral Immunity

- For vaccines that are proposed to protect because they induce humoral immunity, what type of assay should be used (neutralization?) and against what targets (e.g., a panel of HIV viruses, the vaccine immunogen)?
- If neutralization potency must be demonstrated against a panel of viruses/malaria immunogens, how will specifications be set (must similar quantitative values be obtained with each lot for each member of the panel?)

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Measuring Cellular Immunity

- **For vaccines that are proposed to protect because they induce cellular immunity, which assay should be used?**
- **Against what targets/antigens (e.g., multiple malaria proteins; multiple clades of HIV; HIV, TB, and malaria antigens for multi-valent products)?**
- **How quantitative are these assays ("suitably" as defined by the International Conference on Harmonisation in their Q5C and Q6B documents)?**

Wyeth Research

US requirements vs. EU, ROW

- **Plea for harmonization and technically reasonable standards.**

- **We will conduct our potency assays so that we can conduct our studies around the world.**

Wyeth Research

Acknowledgements - HIV Vaccine Programs

WYETH

Steve Udem
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DNA Vaccines Team
Animal Modeling Team
Vaccine Development
Drug Safety & Metabolism
Virus Seed Dev. Team
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Siew Chong
Rashed Abdulla
Shakuntala Megati
Murine Immunobiology
Dave Cooper
Kevin Wright
Priscilla Calderon
Min Guo
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David Clarke
Erik Johnson
Farooq Nasar
Maggie Lee
Sannyu Heron
Viral Vector Team
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Sue Witko
Cheryl Kotash
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John Coleman
Eleanor Ogin-Wilson

YALE
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Rose Lab

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HVTN